

Dopaminergic neuromodulation of prefrontal cortex activity requires the NMDA receptor coagonist D-serine

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Prefrontal control of cognitive functions critically depends upon glutamatergic transmission and N-methyl D-aspartate (NMDA) receptors, the activity of which is regulated by dopamine. Yet whether the NMDA receptor coagonist D-serine is implicated in the dopamine-glutamate dialogue in the prefrontal cortex (PFC) and other brain areas remains unexplored. Here, using electrophysiological recordings, we show that D-serine is required for the fine-tuning of glutamatergic neurotransmission, neuronal excitability, and synaptic plasticity in the PFC through the actions of dopamine at D₁ and D₃ receptors. Using in vivo microdialysis, we show that D₁ and D₃ receptors exert a respective facilitatory and inhibitory influence on extracellular levels and activity of D-serine in the PFC, with actions expressed primarily via the cAMP/protein kinase A (PKA) signaling cascade. Further, using functional magnetic resonance imaging (fMRI) and behavioral assessment, we show that D-serine is required for the potentiation of cognition by D₃R blockade as revealed in a test of novel object recognition memory. Collectively, these results unveil a key role for D-serine in the dopaminergic neuromodulation of glutamatergic transmission and PFC activity, findings with clear relevance to the pathogenesis and treatment of diverse brain disorders involving alterations in dopamine-glutamate cross-talk.

D-serine | NMDA receptors | schizophrenia | D₁- and D₂-type receptors | serine racemase knockout mice

The prefrontal cortex (PFC) supports the higher-order and top-down coordination of complex behaviors, including executive function, working memory, and social interactions (1). Dopaminergic (DAergic) inputs exert a marked influence on the activity of frontocortical circuits, and the dynamic regulation of background dopamine (DA) levels allows for the optimization of PFC cognitive performances (2). Interestingly, DA exerts opposite effects on glutamatergic activity through activation of D₁-type (D₁ and D₅) and D₂-type (D₂, D₃, and D₄) receptors, respectively (3, 4). Specifically, D₁ receptor (D₁R) activation increases neuronal excitability and synaptic efficacy through modulation of N-methyl D-aspartate receptors (NMDARs), while D₂ receptor (D₂R) signaling produces opposite effects (4). Furthermore, activation of D₁R promotes Long-Term Potentiation (LTP) in the PFC (5, 6), whereas D₂R stimulation is required for inducing Long-Term Depression (LTD) (6). These observations support the current view that distinct cortical DA receptors differentially regulate cognitive functions through the modulation of NMDARs. However, the underlying mechanisms still await clarification. While D₁R activation in the PFC consistently

facilitates working memory and executive function (7), the significance of PFC-localized D₂-type receptors is more complex inasmuch as D₂R and D₃R subtypes enact contrasting roles: activation of D₂R favors cognitive processes, whereas D₃R stimulation exerts a negative impact on cognition (8). Accordingly, blockade of D₃Rs results

Significance

Dopamine and glutamate in the prefrontal cortex are important substrates of higher cognitive functions, which are impaired in neuropsychiatric disorders. As regards glutamatergic pathways, a role for the NMDA receptor coagonist D-serine has been highlighted, yet its relationship to dopaminergic transmission remains unclear. In this study, we reveal that D-serine plays a pivotal role in the modulation by dopamine of NMDA receptor activity and cognitive performance in the prefrontal cortex. Comprehensive evidence for this interaction is provided at the synaptic, neuronal, network, and behavioral levels. These observations are of relevance to the pathophysiology and treatment of cognitive impairment in numerous disorders involving disruption of the frontocortical dialogue between dopamine and glutamate.

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in a broad-based positive influence upon cognition, including improvements in social novelty discrimination and novel object recognition (NOR) and a reduction in the cognitive deficits associated with a developmental model for schizophrenia (SCZ) (9, 10). Moreover, it has been proposed that DAR and NMDARs can form clusters at the surface of neurons (11) and that their reciprocal dialogue relies on intracellular signaling cascades involving protein kinases (12). Whether functional DA–NMDAR cross-talk involves others mechanisms is not known.

Activation of the canonical GluN1–GluN2 containing NMDARs requires binding of both glutamate and a coagonist, either glycine or D-serine (13). We have previously shown that D-serine rather than glycine is the primary endogenous coagonist of synaptic NMDARs at glutamatergic synapses connecting pyramidal neurons in the PFC of adult rats (14). This is important since genetic linkage and association studies show that the genes encoding the

D-serine-producing and degrading enzymes serine racemase (SR) and D-amino acid oxidase (DAAO), respectively, are risk genes for SCZ (15, 16). Accordingly, serum and cerebrospinal fluid levels of D-serine are reduced in SCZ patients (16–18). Moreover, clinical trials of D-serine supplementation or the administration of DAAO inhibitors have yielded encouraging results (17, 18). An involvement of D-serine in SCZ is further supported by animal studies showing that multiple risk pathways for SCZ converge in SR-deficient mice (19). Yet, our understanding of the significance of D-serine to the pathology and symptoms of SCZ remains limited, and the question of how it interacts with DA signaling remains to be resolved. This issue is, moreover, of broader pertinence in view of accumulating evidence that a malfunction of NMDAR is central to the pathophysiology of multiple brain disorders (20).

In the current study, we sought to determine whether and how D-serine and DA interact in the PFC. We show that D-serine is

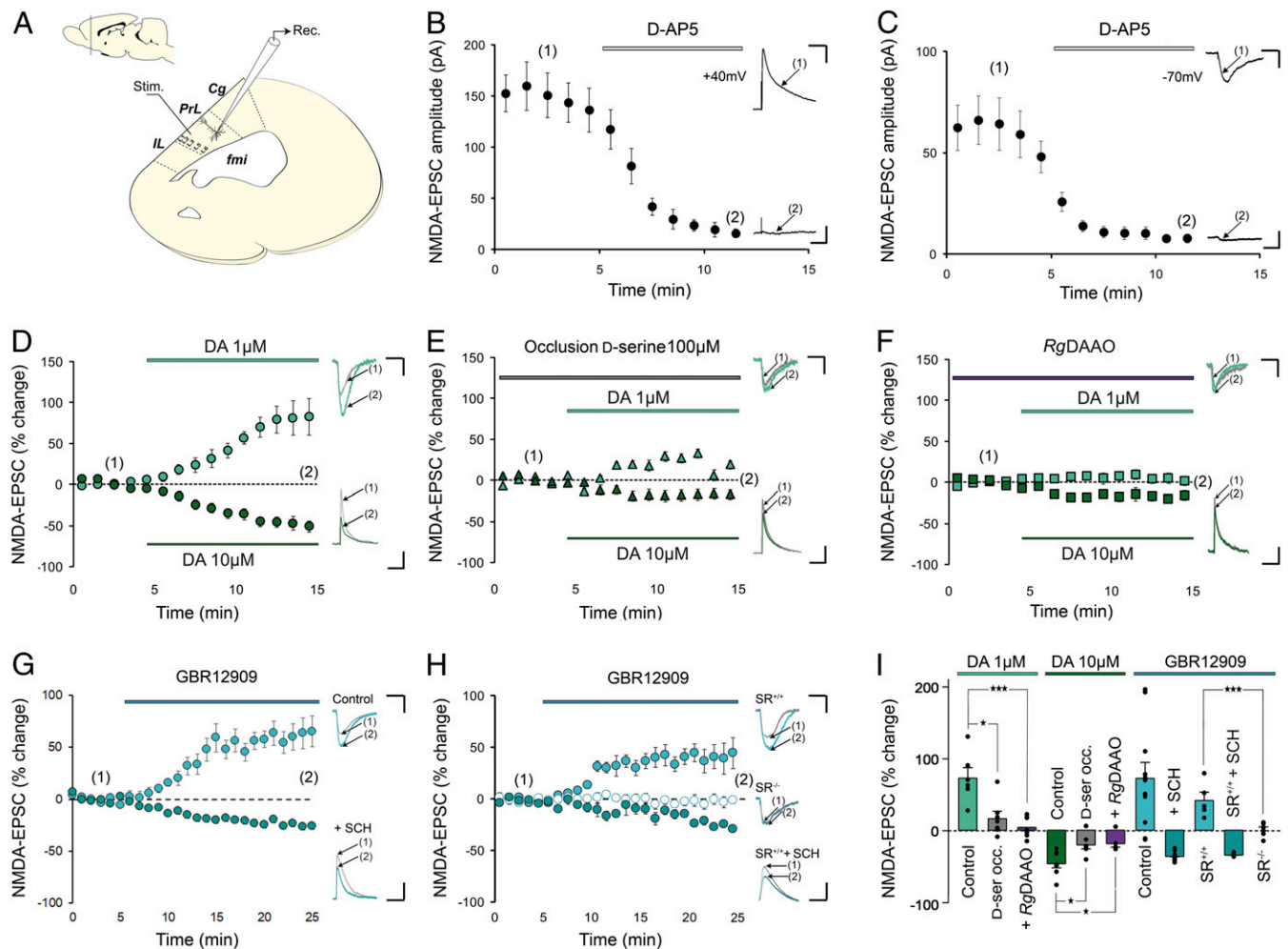


Fig. 1. D-serine is required for dopamine modulations of NMDA-EPSCs. (A) Diagram showing the slice and electrodes placements for recording NMDA-EPSCs at pyramidal neurons in layer 5 of the PRL cortex. (B and C) Pharmacologically isolated NMDA-EPSCs can be recorded in PRL neurons from rat slices at 40 mV and at –70 mV in the presence of GABA-receptor blocker PTX (50 μM) and AMPA-receptor blocker NBQX (10 μM) and are blocked by D-AP5 (50 μM). (D) Bath application of low (light green, 1 μM, $n = 6$) or high (dark green, 10 μM, $n = 7$) concentrations of DA respectively up- (73.9 ± 14.1%) or down-regulate (–44.7 ± 6.7%) NMDA-EPSCs. (E and F) Preincubation with 100 μM D-serine (triangle symbols) or treatment with RgDAAO (square symbols) opposes occluding action on the positive (DA₁ μM + D-Ser: 22.5 ± 4.6%, $n = 7$; DA₁ μM + RgDAAO: 5.4 ± 5.93%, $n = 7$) and negative (DA₁₀ μM + D-Ser: –18.7 ± 6.7%, $n = 6$; DA₁₀ μM + RgDAAO: –16.8 ± 6.2%, $n = 5$) regulations of NMDA-EPSCs by exogenous DA. (G) Accumulation of endogenous DA by application of the DA reuptake inhibitor GBR12909 (0.5 μM) increases NMDA-EPSCs in control rat slices (filled light green circles, 73.78 ± 21.35%, $n = 11$) and decreases NMDA-EPSCs in the presence of the D1R antagonist SCH39166 (1 μM) (filled dark green circles, –35.68 ± 1.45%, $n = 13$). (H) GBR12909 (0.5 μM) increases NMDA-EPSCs in mouse SR^{+/+} control slices (filled light green circles, 41.73 ± 12.12%, $n = 5$) and decreases NMDA-EPSCs in the presence of the D1R antagonist SCH39166 (1 μM) (filled dark green circles, –22.84 ± 1.79%, $n = 5$). GBR12909 (0.5 μM) induces no change in slices from SR^{–/–} mice (empty green circles, 0.93 ± 4.22%, $n = 6$). Top traces scale bars: 50 pA, 100 ms. Bottom traces scale bars: 50 pA, 500 ms. (I) Histograms summarizing the DA modulations of NMDA-EPSCs and the opposing action of D-serine binding site occlusion, RgDAAO treatment, and SR deletion. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

indeed required for the DAergic modulation of NMDARs in the PFC, as expressed in measures of neuronal excitability, synaptic plasticity, and cognitive function, providing a framework for understanding PFC integrated DA–glutamate cross-talk under physiological and pathological conditions.

Results

Modulation of NMDAR and Neuronal Excitability by DA Involves D-Serine. To test the hypothesis that D-serine may be implicated in DA modulation of NMDAR functions, we first investigated whether DA modulations of isolated synaptic NMDAR-mediated excitatory postsynaptic currents (NMDA-EPSCs) recorded at 40 mV and -70 mV in adult rat prelimbic cortex (PrL) layer 5 pyramidal neurons (Fig. 1 *A–C*) require D-serine. Low concentrations of DA ($1\ \mu\text{M}$) potentiated NMDA-EPSCs by $73.9 \pm 14.1\%$ (Fig. 1 *D* and *I* and *SI Appendix*, Fig. S8) while higher concentrations ($10\ \mu\text{M}$) decreased NMDA-EPSCs by $44.7 \pm 6.7\%$ (Fig. 1 *D* and *I* and *SI Appendix*, Fig. S8), confirming previous studies (21, 22). To determine whether these bidirectional modulations of NMDA-EPSCs by DA imply changes in the occupancy of NMDARs by a coagonist, we monitored the effects of DA after presaturation of the coagonist binding site by exogenous D-serine ($100\ \mu\text{M}$) (Fig. 1 *E* and *I*). This resulted in the occlusion of DA-effected NMDA-EPSC modulation. We then directly addressed the implication of D-serine in the DA modulation of NMDA-EPSCs by treating the slices with *Rhodotorula gracilis* D-amino acid oxidase (*RgdAAO*) ($0.2\ \text{U/mL}$) to specifically deplete endogenous D-serine (14, 23) (Fig. 1 *F* and *I*). Akin to the effect of saturating the D-serine binding site, removal of D-serine with *RgdAAO* markedly reduced the potentiating and inhibitory effect of 1 and $10\ \mu\text{M}$ DA, respectively. As an important control, we also confirmed, using enzymatic assays, that DA is not a substrate or an inhibitor for *RgdAAO* (*SI Appendix*, Fig. S1). These data therefore indicate that positive and negative modulations of NMDAR by exogenous DA requires D-serine.

We next assessed whether D-serine is involved in the regulation of NMDA-EPSCs by endogenous DA. To this end, we first studied the effects of the DA reuptake inhibitor GBR12909 on NMDA-EPSCs. Bath-applied GBR12909 ($0.5\ \mu\text{M}$) potentiated NMDA-EPSCs (Fig. 1 *G* and *I*) similarly to exogenous low DA ($1\ \mu\text{M}$). Remarkably, performing this experiment under D₁R blockade by addition of the antagonist SCH39166 ($1\ \mu\text{M}$) not only abolished the potentiating effect of GBR12909 but also unmasked a depressant action on NMDA-EPSCs (Fig. 1 *G* and *I*). To directly address whether the potentiating effect of GBR12909 requires D-serine acting on NMDARs, we next recorded NMDAR currents on PFC slices isolated from mice lacking the D-serine-producing enzyme SR ($\text{SR}^{-/-}$), in which D-serine content is $\sim 10\%$ of wild-type ($\text{SR}^{+/+}$) mice (24). Bath application of GBR12909 ($0.5\ \mu\text{M}$) potentiated NMDA-EPSCs in $\text{SR}^{+/+}$ control slices and depressed these currents upon blockade of D₁Rs. In contrast, in slices from $\text{SR}^{-/-}$ mice, GBR12909 failed to induce any changes in NMDA-EPSCs (Fig. 1 *H* and *I*). Together, these results indicate that D-serine is required for appropriate DA modulation of NMDA-EPSCs.

As DA is known to regulate PFC activity through modulation of pyramidal neurons excitability (4) and modulation of NMDARs also influences PFC neuronal firing activity (25, 26), we next investigated whether D-serine is also involved in the DA modulation of layer 5 pyramidal neurons excitability. To this end, cell firing was induced by incremental square depolarizing current pulses during whole-cell current-clamp recordings. DA concentrations of 1 and $10\ \mu\text{M}$ applied to rat PFC slices respectively increased and decreased neuronal excitability (*SI Appendix*, Fig. S2 *A* and *B*) without altering membrane properties (*SI Appendix*, Table S1). Depletion of D-serine with *RgdAAO* prevented these changes in excitability (*SI Appendix*, Fig. S2 *C* and *D*). We also undertook these experiments in PFC slices from $\text{SR}^{-/-}$ mice and $\text{SR}^{+/+}$

mice. DA at 1 and $10\ \mu\text{M}$ induced changes in $\text{SR}^{+/+}$ mice cell excitability similar to those observed in rats, without altering membrane properties (*SI Appendix*, Table S2 and Fig. S2 *E* and *F*). Absence of D-serine in $\text{SR}^{-/-}$ mice resulted in a complete loss of these DA modulations, mirroring the effect observed with *RgdAAO* (*SI Appendix*, Fig. S2 *G* and *H*). These results thus indicate that D-serine is required for DA-induced changes in NMDAR-dependent neurotransmission and excitability at PFC pyramidal neurons.

Opposite Influence of D₁R and D₃R Activation on NMDAR. Positive DA modulation on PFC pyramidal neurons is classically attributed to D₁-type receptor activation, whereas negative modulation is thought to be mediated by the D₂-type receptor (3). We then tested whether modulation of NMDA-EPSCs by selective D₁-type and D₂-type agonists required D-serine. As D₃Rs were recently found to play an important regulatory role in the PFC and in SCZ (27), we ensured that we could detect these receptors in patched PFC neurons using single-cell RT-PCR (Fig. 2*A*) and particularly focused on this subtype of receptor. Bath application of the preferential D₃R agonist PD128907 ($1\ \mu\text{M}$) reduced NMDA-EPSCs, whereas the D₁R agonist SKF81297 ($10\ \mu\text{M}$) induced a reliable potentiation of NMDA-EPSCs (Fig. 2 *C* and *E*). As with DA, actions of these two agonists were strongly impaired by saturating the NMDARs coagonist binding site with exogenous D-serine ($100\ \mu\text{M}$, Fig. 2 *D* and *E*). Furthermore, application of S33084 ($0.1\ \mu\text{M}$), a selective D₃R antagonist (10), fully abolished the inhibitory effect of PD128907 on NMDA-EPSCs but did not alter the potentiating effect of the D₁R agonist SKF81297 (*SI Appendix*, Fig. S3).

We next analyzed the effects of D₁- and D₃R agonists on NMDA-EPSCs in $\text{SR}^{-/-}$ mice. Mirroring rat PFC slices, application of SKF81297 and PD128907 respectively increased and decreased NMDAR-EPSCs in $\text{SR}^{+/+}$ slices (Fig. 2 *F* and *H*), and these effects were markedly compromised in $\text{SR}^{-/-}$ slices (Fig. 2 *G* and *H*). To further confirm that synaptic deficits in $\text{SR}^{-/-}$ mice were specifically attributable to a lack of D-serine and not to other putative modulators such as glycine or D-aspartate, we restored its levels in $\text{SR}^{-/-}$ mice by chronic subcutaneous administration ([D-serine] = $150\ \text{mg/kg}$) for 21 d, as previously described (19). While levels of D-serine, D-aspartate, and glycine in the medial PFC (mPFC) were respectively decreased by $\sim 89\%$, $\sim 35\%$, and $\sim 69\%$ in saline-treated $\text{SR}^{-/-}$ mice, treatment with D-serine selectively re-established levels of D-serine alone to near-control values in mutant mice (*SI Appendix*, Fig. S4). Importantly, this resulted in the normalization of the modulatory actions of D₁R and D₃R agonists on NMDA-EPSCs in D-serine-treated but not in saline-treated $\text{SR}^{-/-}$ mice (Fig. 2 *I–K*). Thus, these rescue experiments confirm that in $\text{SR}^{-/-}$ mice, D-serine is the missing element affecting DAergic modulation of NMDARs. Collectively, these experiments demonstrate that D-serine is a critical factor for DA modulation of NMDARs via D₁- and D₃Rs.

D-serine Mediates DA Modulations of Synaptic Plasticity. Activity-dependent changes of synaptic strength in the PFC rely on NMDARs (6, 14) and are strongly regulated by DA (3, 5, 6). In particular, D₁-type receptor stimulation enhances LTP while LTD requires the activation of both D₁- and D₂-type receptors (5, 6). To specifically determine the contribution of D-serine to DA modulation of NMDAR-dependent long-term changes in synaptic plasticity, we investigated the effect of DA receptor stimulation on excitatory postsynaptic field potential plasticity in the PrL of $\text{SR}^{+/+}$ and $\text{SR}^{-/-}$ mice. Analyses of $\text{SR}^{-/-}$ mice synaptic function revealed that basal synaptic transmission was reduced by $\sim 26\%$ (Fig. 3*A*), a decrease most likely attributable to postsynaptic mechanisms as paired-pulse experiments did not show any changes in release probability (Fig. 3*B*). These results are reminiscent of previously reported NMDAR hypofunction in $\text{SR}^{-/-}$ mice (19, 24). Although

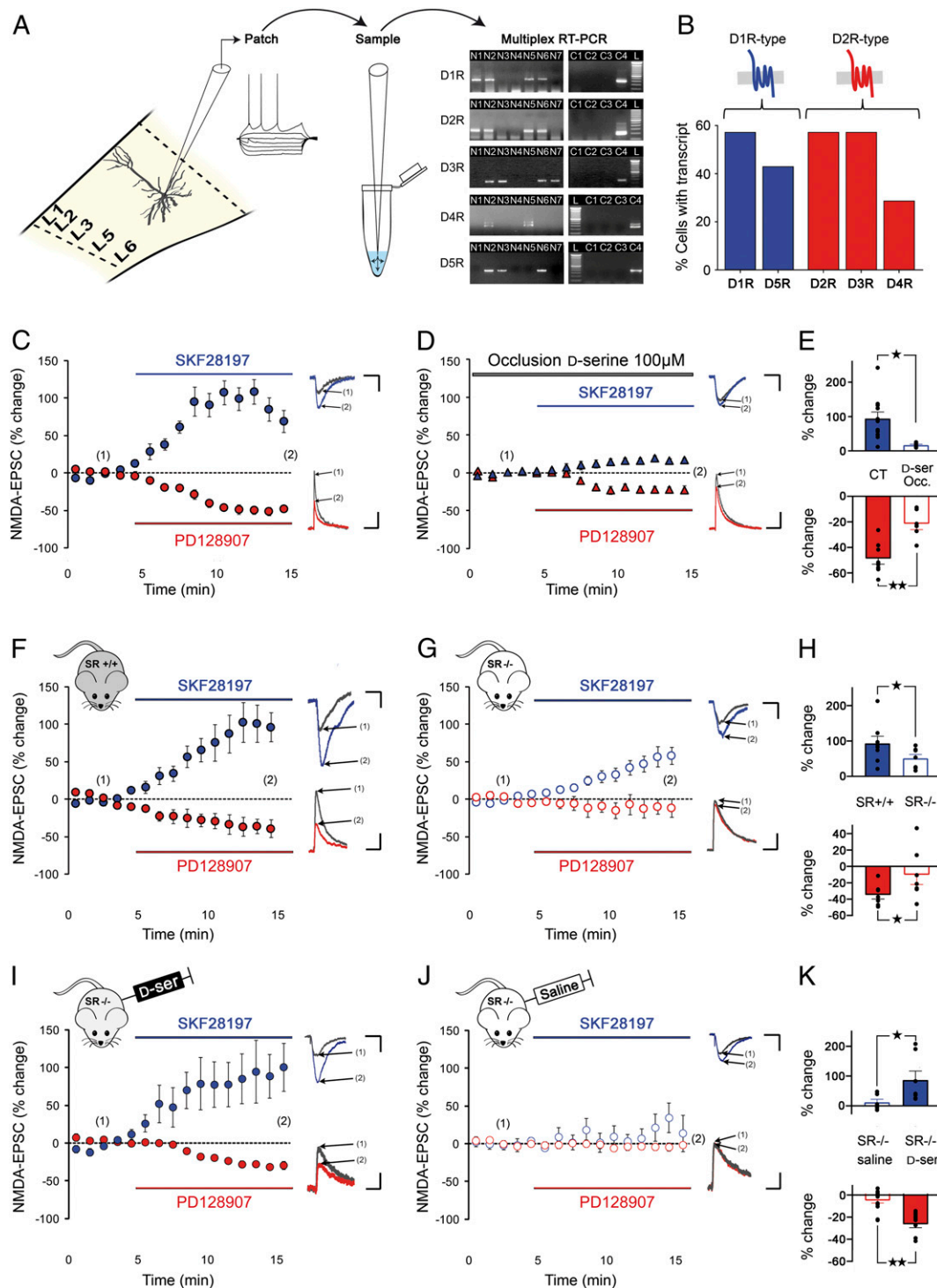


Fig. 2. Modulations of NMDA-EPSCs by D₁- and D₃-receptors involves D-serine. (A and B) Single-cell multiplex RT-PCR showing the expression of D1 and D2 receptors subtypes in L5 excitatory neurons of the PrL area. (C) Bath application of D₁R agonist SKF81297 10 μ M increases NMDA-EPSCs ($93.93 \pm 14.05\%$, $n = 11$) while the D₃R specific agonist PD128907 1 μ M has an opposing action ($-39.67 \pm 8.1\%$, $n = 8$). (D) Occluding the coagonist binding site of NMDAR with 100 μ M D-serine markedly impairs NMDAR-EPSCs modulations by DA receptors activation (SKF81297+D-ser: $17.04 \pm 2.48\%$, $n = 6$; PD128907+D-ser: $-21.7 \pm 5.6\%$, $n = 6$). (E) Histograms summarizing the effects of D₁R and D₃R agonists in the absence and presence of occluding D-serine. (F) Modulations of NMDAR-EPSCs by D₁Rs and D₃Rs activation in SR^{+/+} mice (SKF81297: $92.66 \pm 23.5\%$, $n = 8$; PD128907: $-34.92 \pm 4.95\%$, $n = 7$). (G) Modulations of NMDAR-EPSCs by D₁Rs and D₃Rs activation in SR^{-/-} mice (SKF81297: $51.03 \pm 10.91\%$, $n = 7$; PD128907: $-10.36 \pm 11.75\%$, $n = 7$). (H) Histograms showing that modulations of NMDAR-EPSCs by D₁Rs and D₃Rs activation are impaired in SR^{-/-} mice. (I) Modulations of NMDAR-EPSCs by D₁Rs and D₃Rs activation in SR^{-/-} mice chronically supplemented with D-serine (SKF81297: $86.86 \pm 30.01\%$, $n = 7$; PD128907: $-26.46 \pm 4.19\%$, $n = 14$). (J) Modulations of NMDAR-EPSCs by D₁Rs and D₃Rs activation in SR^{-/-} mice chronically supplemented with control saline (SKF81297: $11.98 \pm 10.32\%$, $n = 6$; PD128907: $-3.70 \pm 4.56\%$, $n = 14$). (K) Histograms showing that chronic D-serine supplementation rescues modulations of NMDAR-EPSCs by D₁Rs and D₃Rs activation in SR^{-/-} mice. * $P < 0.05$; ** $P < 0.01$. Top traces scale bars: 50 pA, 100 ms. (Bottom traces scale bars, 50 pA, 500 ms.)

no difference in LTP magnitude between $SR^{+/+}$ and $SR^{-/-}$ mice was detectable (Fig. 3C), D₁-type receptor activation by SKF81297 (10 μ M) enhanced LTP in $SR^{+/+}$ (Fig. 3D), consistent with previous investigations (5, 6), but not $SR^{-/-}$ mice (Fig. 3E), thereby indicating that D-serine is required for DAergic modulation of PrL LTP.

We then investigated LTD. As previously reported (6), low-frequency stimulation (LFS) could only induce LTD when performed in conjunction with D₁- and D₂-type receptor activation (SI Appendix, Fig. S5 and Fig. 3F). Indeed, combining SKF81297 (10 μ M) and quinpirole (1 μ M) with LFS elicited LTD (SI Appendix, Fig. S5D). Strikingly the latter effect was abolished in D₃R knockout ($D_3R^{-/-}$) mice (SI Appendix, Fig. S5D), indicating that D₃Rs play a critical role in the DA-dependent generation of LTD in the PrL. Accordingly, costimulation of D₁R and D₃R with SKF81297 and PD128907 combined with LFS reliably induced LTD in $SR^{+/+}$ mice (Fig. 3F). Importantly, this DA-dependent LTD was markedly reduced by $\sim 62\%$ in $SR^{-/-}$ mice (Fig. 3F). Collectively, these data indicate that D-serine is critical for the DAergic modulation of long-term synaptic plasticity in the PrL via both D₁R and D₃R.

Activation of D₁R and D₃R Modulates PFC D-serine Levels In Vivo. To gain insights into how D-serine participates in the DAergic modulations of NMDAR function, we tested whether activation of D₁- and D₃Rs could regulate extracellular levels of this NMDAR coagonist. To this end, we performed bilateral in vivo microdialysis in the mPFC of freely moving mice measuring basal extracellular levels of D- and L-serine for 1 h in response to perfusion of artificial cerebrospinal fluid (aCSF) as a control, the D₁R agonist SKF81297 (10 μ M), the D₃R agonist PD128907 (1 μ M), or a combination of both (Fig. 4A–C). The basal levels of D-serine and L-serine in the mPFC were 812.80 ± 22.97 nM and $1,514.00 \pm 126.30$ nM, respectively, in the range of previous studies (28, 29). SKF81297 increased extracellular levels of D-serine level by $\sim 17\%$, providing a mechanism for the D-serine-dependent activation of NMDARs by D₁Rs reported herein. Although activation of D₃Rs with PD128907 alone did not induce any change in extracellular D-serine levels, it blunted the increase in D-serine levels elicited by D₁R activation (Fig. 4C, Left), indicating functional cross-talk between D₁R and D₃R. Interestingly, local infusion of SKF81297 also increased levels of L-serine, the precursor of D-serine, and this effect was also blunted by D₃Rs activation (Fig. 4C, Right). These data therefore indicate that D₁R and D₃R activation

modulate extracellular levels of D-serine via direct release as well as increased biosynthesis by SR and/or decreased degradation by DAAO. Since we found that chronic supplementation of D-serine was sufficient to restore D₁R and D₃R modulations of NMDARs in $SR^{-/-}$ mice, we conclude that release of D-serine is the primary process at play.

To ascertain whether the inhibitory influence of D₃R on D₁R is indeed functionally relevant at NMDARs, we recorded NMDA-EPSCs in the presence of PD128907 (1 μ M) and under these conditions applied SKF81297 (10 μ M). Instead of potentiating NMDA-EPSCs as occurs when applied alone (Fig. 2F), SKF81297 in the presence of PD128907 induced no change (Fig. 4D), thus corroborating the inhibitory action that D₃R activation exerts on D₁R function. These data suggest that D-serine function at synaptic NMDARs can be fine-tuned by DAergic input via the balanced activation of D₁- and D₃Rs (Fig. 4D).

The Balance of D₁ versus D₃R Activation Regulates D-serine Functions via the cAMP/PKA Pathway. We next sought to determine the signaling cascade underlying the D₁/D₃R cross-talk regulating D-serine function. To this end, we focused on the adenylyl cyclase (AC)–cAMP–protein kinase A (PKA) pathway, because AC activity is differentially influenced by both D₁- and D₃R (3) and PKA is known to regulate exocytosis (30, 31). We posited that D₁R activation leads to an up-regulation of AC activity to induce an increase in D-serine release, whereas D₃Rs activation inhibits this process by suppression of AC. To test this hypothesis, we inhibited AC with SQ22536 (20 μ M) (Fig. 5A) or PKA with H89 (10 μ M) (Fig. 5B) in $SR^{+/+}$ PFC slices and under these conditions activated D₁Rs with SKF81297 (10 μ M). Blocking AC or PKA yielded a similar blunting effect on D₁R-induced facilitation of NMDA-EPSCs (Fig. 5A and B) as activation of D₃Rs (Fig. 4D), thus corroborating our hypothesis. Accordingly, blockade of PKA also occluded the effect of D₃R activation (Fig. 5C). To directly test whether this signaling pathway is indeed underlying DAergic modulation of D-serine functions, we assessed the effect of AC activation with forskolin (50 μ M) on $SR^{+/+}$ and $SR^{-/-}$ NMDA-EPSCs. While, akin to D₁R stimulation, AC activation induced a robust increase in NMDA-EPSCs amplitude in $SR^{+/+}$ mice ($57.60 \pm 10.94\%$), this effect was abolished in $SR^{-/-}$ mice (Fig. 5D). Together these data identify the AC–cAMP–PKA signaling cascade as a key intracellular pathway transducing the opposing actions of D₁R and D₃R on D-serine function.

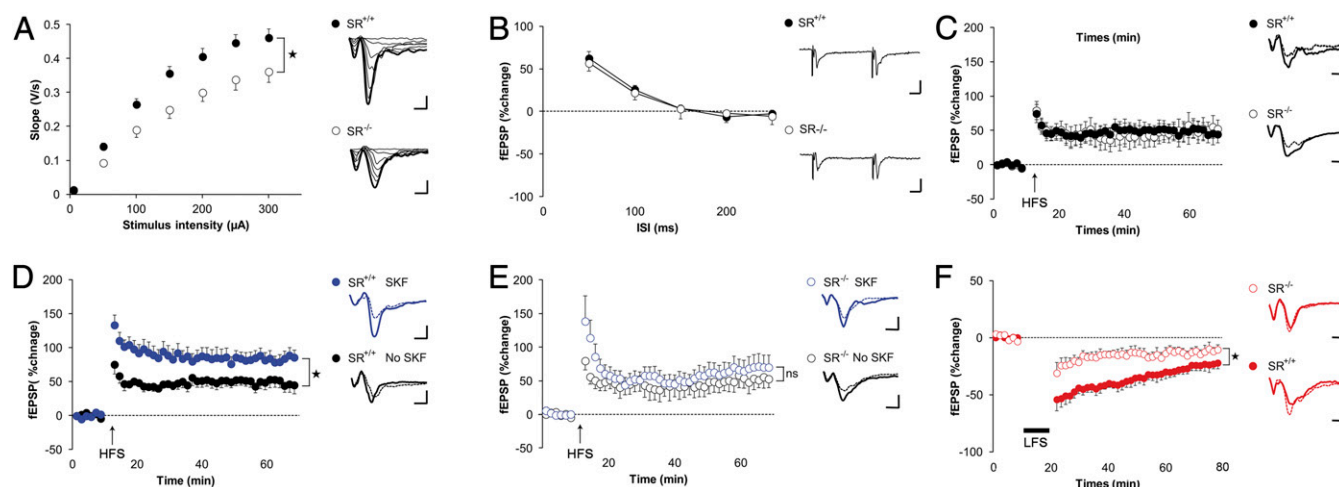


Fig. 3. D-serine mediates DA regulations of synaptic plasticity. (A) Input/output relationship in wild-type versus SR-deficient mice ($SR^{+/+}$ $n = 44$; $SR^{-/-}$ $n = 21$). (B) Paired-pulse ratio profiles in wild-type versus SR-deficient mice ($SR^{+/+}$ $n = 44$; $SR^{-/-}$ $n = 21$). (C) LTP of the field potential in wild-type versus SR-deficient mice ($SR^{+/+}$: $48.88 \pm 9.20\%$, $n = 9$; $SR^{-/-}$: $48.13 \pm 14.6\%$, $n = 8$). (D) Effect of the D₁-type agonist SKF81297 (10 μ M) on LTP in $SR^{+/+}$ mice ($83.66 \pm 10.72\%$, $n = 10$). (E) Effect of SKF81297 (10 μ M) on LTP in $SR^{-/-}$ mice ($61.64 \pm 14.66\%$, $n = 9$). (F) LTD induced by concomitant application of SKF81297, PD128907, and LFS in wild-type versus SR-deficient mice ($SR^{+/+}$: $-32.39 \pm 7.58\%$, $n = 6$; $SR^{-/-}$: $-12.20 \pm 5.44\%$, $n = 6$). * $P < 0.05$. (Scale bars, 0.1 mV, 2 ms.)

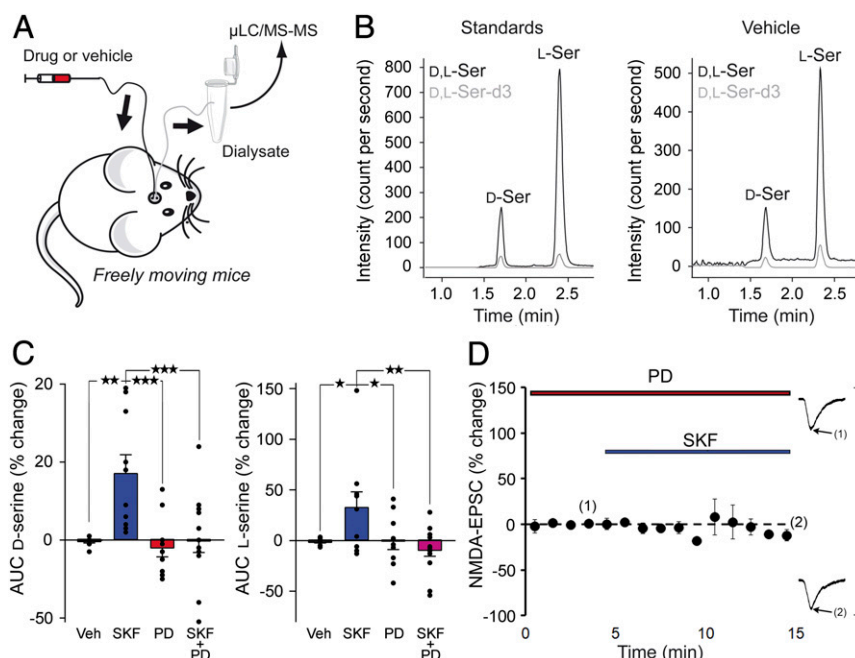


Fig. 4. DA receptors activation regulates extracellular mPFC D-serine levels in freely moving mice. (A) Mice were implanted with two microdialysis probes placed in the left and right mPFC. Microdialysates were collected every 20 min and D- and L-serine were quantified using UHPLC-MS/MS. (B) Left chromatogram shows separation of a standard mixture of D-serine at lower limit of quantification (LLOQ) (20 nM) and L-serine in amino acid standard 18 in artificial cerebrospinal fluid (black line) and D,L-serine-d3 (230 nM, internal standard, gray line). Right chromatogram shows a typical microdialysis sample in basal conditions (vehicle, black line) and D,L-serine-d3 (gray line). (C) Changes in extracellular D-serine and L-serine levels from 20 to 60 min during drug infusion in the mPFC. Histograms represent values for area under the curve (AUC). (Left) The D₁R agonist SKF81297 increased the extracellular levels of D-serine by $17.40 \pm 4.73\%$ ($n = 8$ samples, 6 mice) while D₃R agonist PD128907 on its own had no effect (vehicle: $-0.25 \pm 0.49\%$ change, $n = 10$ samples from six mice versus PD128907: $-1.90 \pm 2.4\%$ change, $n = 10$ samples from six mice) but blocked the potentiating effect of SKF81297 ($-0.08 \pm 3.13\%$ change, $n = 13$ samples from seven mice). (Right) SKF81297 also increased the extracellular levels of L-serine by $33.10 \pm 15.05\%$ while D₃R agonist PD128907 had no effect on its own (vehicle: $-1.31 \pm 1.15\%$ change versus PD128907: $0.00 \pm 8.90\%$ change) but blocked the potentiating effect of SKF81297 ($-9.15 \pm 6.32\%$ change). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. (D) Recordings of NMDA-EPSCs in the mPFC of mice showing that in the presence of PD128907, SKF81297 fails to potentiate NMDA responses ($-3.15 \pm 6.16\%$ change, $n = 6$).

DAergic Modulation of PFC Cognitive Functions Involves D-serine. To evaluate the implication of this newly identified neuromodulatory interplay on cognition, we next investigated the role of D-serine in D₃R modulation of PFC-dependent cognitive processes. We previously showed that antagonizing D₃Rs with systemic administration of S33084 (0.63 mg/kg) enhances rat short-term memory in the NOR task (10). Here, we further established that this effect was attributable to changes in PFC function by testing NOR memory in rats after intra-PFC administration of S33084 5 min prior to the acquisition phase (T1, *SI Appendix, Fig. S6A*). As with systemic administration, treatment resulted in the retention of the novel object 4 h after T1, while in control vehicle-treated rats, NOR memory was lost at this intertrial interval (ITI) (10), *SI Appendix, Fig. S6B*, thus confirming the PFC origin of the procognitive effect of D₃R blockade on recognition memory.

To test the involvement of NMDARs in this procognitive effect, the noncompetitive NMDAR channel blocker MK801 (0.05 mg/kg, Intraperitoneal injection [i.p.]) or the selective and competitive NMDAR antagonist CPP (10 mg/kg, i.p.) were administered 15 min prior to S33084 (0.63 mg/kg, subcutaneous [s.c.]). Both NMDAR blockers prevented the procognitive effect of D₃R antagonism on NOR memory (Fig. 6A). We also studied the contribution of the coagonist modulatory binding site using the selective D-serine/glycine binding site antagonist, L701,324 (5 mg/kg, i.p.) (32), which also abolished the reversal of delay-induced impairment in NOR memory by S33084 (Fig. 6A). These results indicate that the procognitive action of S33084 depends on NMDAR function and activation of the coagonist site, possibly via D-serine.

To further explore this possibility, we assessed NOR memory in SR^{+/+} and SR^{-/-} mice. The two genotypes displayed similar

baseline NOR performances, being intact at 2 min and 1 h after the acquisition trial but lost after a 2-h ITI (*SI Appendix, Fig. S6C*). Thus, a deficit in D-serine does not affect NOR performance per se. However, S33084 applied systemically (0.63 mg/kg, s.c.) 30 min before T1 or by local intra-PFC injection (2.5 μg, 0.5 μL) 5 min prior to T1 reversed the 2-h delay-induced NOR impairment in SR^{+/+} mice (Fig. 6B and *SI Appendix, Fig. S6D*). This result indicates that in mice, D₃R antagonism also yields a procognitive action that relies on PFC neuromodulation. Strikingly, S33084 (0.63 mg/kg, s.c.) was ineffective in SR^{-/-} mice (Fig. 6B), thus demonstrating the pivotal role of D-serine in the DA modulation of PFC-dependent cognitive functions. We here further ensured that this impairment was indeed attributable to the lack of D-serine in SR^{-/-} mice by performing rescue experiments in which D-serine supplementation of SR^{-/-} mice reversed this deficit (Fig. 6B). Taken together, these data demonstrate that, in the PFC, modulation of D-serine function via D₃Rs impacts cognition.

DAergic Regulation of PFC Global Activity Is Impaired in SR Knockout Mice. We finally tested in vivo whether D₃R modulation of PFC activity is impaired in SR^{-/-} mice by pharmacological magnetic resonance imaging (phMRI). Since antagonizing D₃Rs potentiates the cerebral blood volume response (rCBV) to the DA-releasing compound D-amphetamine in cingulate cortex (33), we reasoned that if D-serine is required for optimal DAergic modulation of PFC activity, the rCBV change in response to D-amphetamine in the presence of S33084 (0.63 mg/kg, s.c.) should be impaired in SR^{-/-} mice. As predicted, SR^{-/-} mice displayed a blunted rCBV response to D-amphetamine (1 mg/kg, i.p.), as compared with control SR^{+/+} mice (*SI Appendix, Fig. S7 A–C*). These data therefore confirm

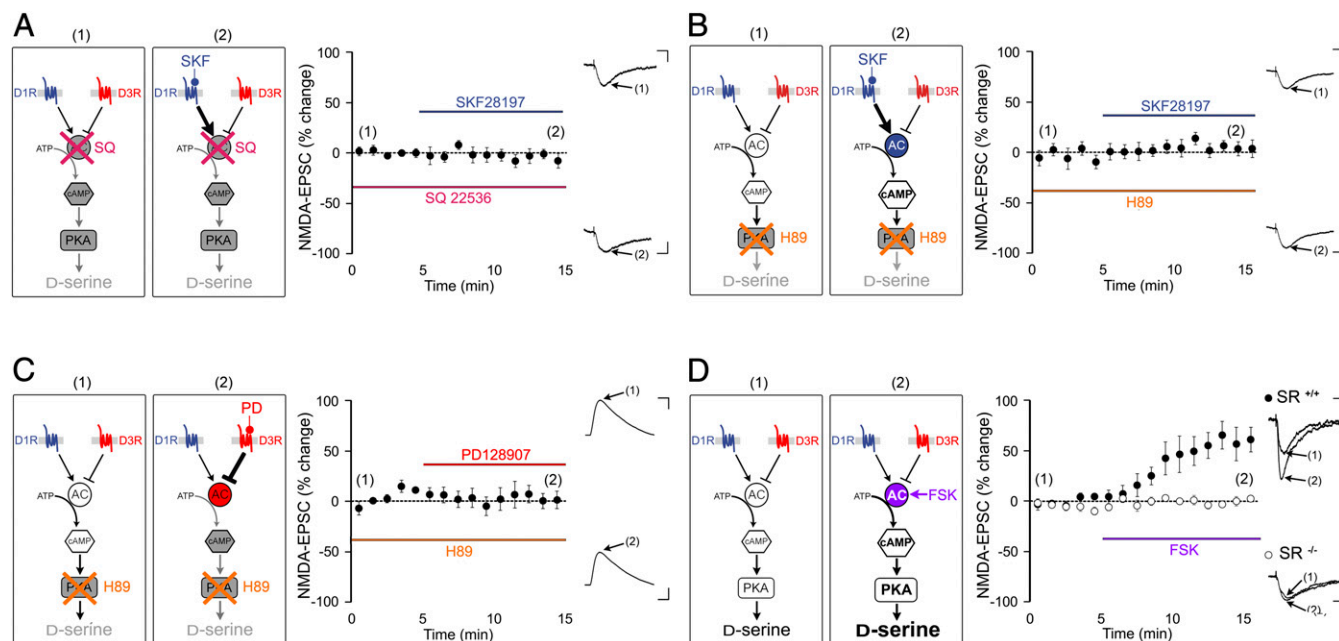


Fig. 5. DA modulations of D-serine NMDAR coagonist functions occurs through cAMP/PKA signaling. (A) The facilitating effect of D₁R activation on NMDA-EPSCs is abolished by blocking production of cAMP by the AC enzyme ($-4.52 \pm 3.21\%$, $n = 12$). (B) Similarly, blocking downstream the PKA enzyme prevents the facilitating effect of D₁R activation on NMDA-EPSC ($6.18 \pm 3.27\%$, $n = 12$). (C) Down-regulation of NMDAR-EPSCs by D₃R activation is also abolished by H89 blockade, which is indicative of an occlusion. (D) Activating AC increased NMDA-EPSCs in SR^{+/+} mice ($51.61 \pm 11.62\%$, $n = 10$) but not in SR^{-/-} mice ($-0.63 \pm 2.01\%$, $n = 9$; $P < 0.001$). (Scale bars, 20 pA, 100 ms.)

in vivo that DA modulation of PFC activity occurs through regulation of D-serine.

Discussion

The present study reveals a role for D-serine in driving PFC dopamine–glutamate interactions at the cellular, network, and behavioral level. The underlying signaling pathway involves differential regulation of AC activity by D₁- and D₃R, most likely resulting in PKA-mediated changes in D-serine release. The pharmacological tools employed in this study were in general used at a single dose which may perhaps be a limitation. Yet we selected doses selective for their respective targets and the use of several agents, together with other complementary approaches, reinforces our conclusions.

Activation of D₁R enhances NMDAR-dependent functions to influence neurotransmission, synaptic plasticity, and cell excitability through D-serine. Conversely, activation of D₃R exerts an opposite and inhibitory action. Although contrasting effects of D₁- and D₂-type receptors on NMDAR currents have been reported to partly act via distinct excitatory and inhibitory microcircuits (34), such a regulatory mode can be ruled out since, in this study, all electrophysiological recordings were performed in the presence of the GABA_A receptor antagonist picrotoxin to block GABAergic inhibitory transmission. Still, the question remains whether the DA modulations of D-serine functions unveiled herein are cell autonomous. We found that activation of D₁R increased extracellular levels of D-serine and, in consequence, NMDAR-dependent functions in the PFC, an effect counteracted by activation of D₃R, indicating functional interactions between the two receptors. Our data further indicate that the underlying mechanism involves differential regulation of the AC-PKA pathway, consistent with a cell-autonomous effect. As previous studies showed that D₁R- and D₃R-positive pyramidal neurons are particularly abundant in L5 of mPFC and that the two receptors may reside on the same neurons (35), such a scenario is indeed conceivable.

Intriguingly, we found that modulation of L-serine levels by D₁- and D₃R mirrored their influence on D-serine. Although these

observations might suggest intensification of the D-serine biosynthesis pathway through activation of the glia-to-neuron serine shuttle (36), the fact that we could rescue DAergic control of synaptic and cognitive functions by D-serine supplementation in SR^{-/-} mice indicates that modulation of D-serine biosynthesis is not the limiting step in DA regulation. Rather, DA-induced changes in neuronal D-serine release are most likely central to these processes, while L-serine shuttling and D-serine synthesis by SR would be secondarily up-regulated as a homeostatic loop to support the metabolic demands imposed on neuronal circuitry.

Modulation of D-serine levels upon activation of D₁- or D₃R offers a multifaceted signaling system for DA fine-tuning of NMDAR function, thereby optimizing synaptic and network activity. Tonic, spontaneous DA release may increase NMDAR function through D₁-type receptors, while the higher extracellular level of DA attained during phasic firing of ventral tegmental area-derived mesocortical pathways would recruit D₃R. By inhibiting the AC-PKA pathway, D₃R would counteract D₁R activation to lower D-serine levels and, as a consequence, the operation of NMDAR. In line with this notion, enhancement and reduction of NMDAR activity by respectively low and high concentrations of DA has previously been reported (21, 22) and is coherent with the U-shaped function of the influence of DA receptors activation upon cognition (37, 38). In the case of PFC, such effects have been attributed to the contrasting level of expression of D₁- and D₂-type receptors (39) as well as the differential localization of these receptors (3, 39). In addition, there is compelling evidence for a differential facilitatory and suppressive influence upon cognitive function of D₁ and D₃R, respectively (27, 38).

The striking defects in D₁R- and D₃R-dependent modulation of neurotransmission and PFC function in SR^{-/-} mice suggest that DA modulation specifically involves NMDAR coagonism by D-serine but not glycine. It might then be inquired why SR^{-/-} mice did not exhibit baseline deficits in the NOR procedure which requires functionally intact NMDARs (40). We propose that under conditions of a persistent lack of D-serine in the mPFC, glycine can, to some extent, assume a compensatory role at NMDARs. Possible

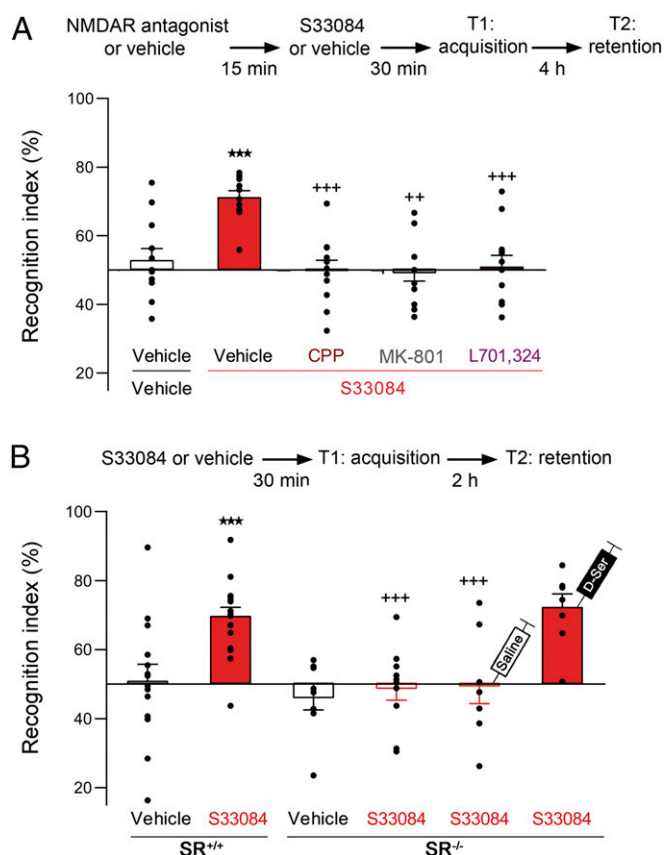


Fig. 6. Procognitive action of D₃R antagonist S33084 relies on D-serine modulation of NMDARs. (A) Administration of the D₃R antagonist S33084 (0.63 mg/kg, s. c.) reverses the impairment in NOR memory induced by a 4-h ITI in rats ($n = 12$). Systemic administration of the NMDAR antagonists, MK801 ($n = 12$) and CPP ($n = 12$), as well as the glycine/D-serine binding site blocker L701,324 ($n = 12$) all prevented the enhancement of NOR memory induced by S33084. (B) Administration of the D₃R antagonist S33084 (0.63 mg/kg, s. c.) reversed the impairment in NOR memory induced by a 2-h ITI in SR^{+/+} mice (vehicle, $n = 14$; S33084, $n = 18$) but not in SR^{-/-} mice (vehicle: $n = 9$; S33084, $n = 10$). The procognitive effect of S33084 is rescued in SR^{-/-} mice by chronic supplementation of D-serine (SR^{+/+}+saline, $n = 7$; SR^{-/-}+D-ser, $n = 8$) performed by s. c. administration with 300 mg/kg D-serine (or control saline) on day 1 and then 150 mg/kg D-serine (or control saline) daily for 20 d. ** $P < 0.01$, *** $P < 0.001$ compared with control SR^{+/+} mice treated with vehicle. ** $P < 0.01$, *** $P < 0.001$ compared with SR^{+/+} mice treated with S33084.

reasons for the specific role of D-serine rather than glycine as a mediator of DA modulation of PFC functions include differential actions related to their spatial segregation. Indeed, we previously found that under physiological conditions, synaptic pools of D-serine but not glycine gate synaptic NMDARs in the mPFC (14), whereas glycine preferentially activates extrasynaptic NMDARs (41).

As for potential pathophysiological relevance, by identifying D-serine as a substrate for the influence of DA upon glutamatergic transmission in PFC, the current observations are relevant to several neuropsychiatric disorders, such as SCZ in which there is a well-established link between a dysfunction of D-serine-mediated NMDAR signaling and both cognitive and positive symptoms (19). Our findings are also consistent with a previous report by Nomura and colleagues showing that neonatal D-serine supplementation of mice displaying a knockout for the SCZ susceptibility gene *pick1* rescues DA modulation of NMDAR-mediated control of pyramidal cell excitability (42). Interestingly, the atypical antipsychotic clozapine enhances D-serine release in rat frontal cortex (43) and elevates plasma levels of D-serine and L-serine in patients with SCZ (44). As clozapine notably acts on DA receptors, in light of our findings, one interpretation might be that clozapine actually changes the balance between D1- and D2-type receptors activation, thereby leading to an increase in D-serine levels. The present study may also offer a cellular explanation for previous studies showing that D-serine plays a major role in cocaine-induced sensitization (45, 46) and to studies suggesting that the novel antipsychotics cariprazine and blonaneserin strengthen cognitive functions by virtue of their attenuation of D₃R activity in the PFC (47, 48). In addition, there is increasing interest in the potential utility for ligands at NMDAR in the management of other disorders from autism to Alzheimer's disease (49, 50).

Finally, gender is clearly a factor related to the prevalence, clinical picture, and treatment of many psychiatric disorders, so it should be pointed out that only males were used in the present study, representing a potential limitation to any clinical extrapolation. Nonetheless, the present study provides an updated framework for an improved understanding and, ultimately, management of the diversity of brain disorders involving disruption of the frontocortical DA-glutamate interface (51, 52).

Methods and Materials

A detailed description of all methods and materials can be found in *SI Appendix*. All experiments were conducted in accordance with European and French directives on animal experimentation and with local ethical committee approval. Electrophysiological recordings and behavioral analyses were performed on adult Wistar and Lister hooded rats as well as SR knockout mice (SR^{-/-}) and control littermates (SR^{+/+}) while in vivo microdialysis and pHMRI experiments were performed only on mice. Only males were used. All quantitative data are expressed as mean \pm SEM. Statistical analyses performed are detailed in *SI Appendix* and in figure legends.

Data Availability. All study data are included in the article and/or *SI Appendix*.

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